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Synthesis and evaluation of 17 α -E-20-(heteroaryl)norpregn-1,3,5(10),20 tetraene-3,17 β -diols [17 α -(heteroaryl)vinyl estradiols] as ligands for the estrogen receptor- α -ligand binding domain (ER α -LBD)

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Abstract

A series of 17 α -(heteroaryl)vinyl estradiols was prepared to evaluate the influence of heteroatom on the affinity and efficacy of estrogenic ligands for the estrogen receptor- α ligand binding domain (ER α -LBD). The products demonstrated reduced binding affinity compared to the parent 17 α -E-phenylvinyl estradiol, but the binding was relatively independent of the heteroatom. The greatest influence of the heteroatom was evident in the efficacy of the compounds as the thienyl derivatives **2f,g** were more potent than either the pyridyl **2b–d** or pyrimidinyl **2e** analogs. The results suggest that a subtle interplay of interactions between the ligands and the receptor influences the biological response.

Keywords

Heteroarylvinyl estadiols; estrogen receptor- α ligand binding domain; competitive binding assay; alkaline phosphatase induction; relative binding affinity; relative stimulatory activity

The estrogen receptor (ER) is a member of the nuclear receptor (NR) superfamily of transcription factors, a group that mediates a wide variety of physiological and developmental processes.^{1–3} Because inappropriate or over-expression of ER is associated with a number of endocrine disorders, such as breast, endometrial and ovarian cancer, and osteoporosis, chemical modulation of the ER-regulated pathways is a critical clinical objective. Recent reviews described the structure of the ER, including its subtypes, and the general mechanism by which binding of the endogenous ligand and its steroidal and nonsteroidal analogs initiate the events leading to transcription (agonist responses) or

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modulation of transcription (antagonism/selective ER modulation).^{4–11} While many individual steps are involved in the overall process, the initial binding of the ligand to the (apo)receptor to generate a stable complex constitutes the key step that defines the subsequent events. Virtually all subsequent biological responses are influenced by the nature of the receptor-ligand complex conformation induced by this initial interaction. Although analysis of the structures of ligand-ER-LBD complexes has provided significantly enhanced understanding of these interactions, the static nature of crystal structures leaves many questions unanswered.^{12–15} Based upon this premise, efforts to characterize the initial step remain important for contributing to the understanding regarding how the subsequent biological consequences are generated.

Our research program has focused on probing the topology of the ER α -LBD through the preparation and evaluation of systematically modified derivatives of estradiol.^{16–23} Such structural probes permit enhanced insight into the influence of physicochemical properties in modulating receptor affinity, selectivity and efficacy. Most of our initial work has focused on the 17 α -position through the use of modifications of the phenylvinyl moiety.^{18–20,22,23} These studies demonstrated that, in general, small substituents on the 17 α -phenylvinyl group were well tolerated (high affinity), but binding was strongly influenced by stereochemistry around the carbon-carbon double bond, and particularly by the position and nature of the moiety on the phenyl group. Moreover, these binding potencies were largely, but not absolutely, translated to observed *in vivo* activity. Of particular note, all of the simple phenylvinyl derivatives, regardless of E-/Z- stereochemistry or ortho-, meta-, para-substitution, were agonists. Molecular modeling studies, coupled with x-ray crystallographic analysis of one of the compounds, complexed with ER α -LBD, indicated that the ligand and the receptor induced mutual adaptive responses to generate an agonist conformation for all of the simple, mono-substituted derivatives.²⁴ Because even small substituents induce changes within the protein structure, we decided to eliminate the pendant groups and modify the nature of the aromatic moiety. Such modifications should reduce the steric factors while generating changes in the electronic character of the aromatic ring. This paper describes the effects of such modifications on the affinity and efficacy of the 17 α -E-heteroarylvinyl estradiols.

Synthesis of 17 α -E-heteroarylvinyl estradiols 2a–g

The synthetic strategy for preparing the target compounds of this study is shown in Scheme 1. Initially we proposed preparing the target compounds via the Suzuki coupling between 17 α -E-iodovinyl estradiol and the corresponding heteroaryl boronic acid. The rationale for this method was based on the reported ease of the Suzuki coupling reaction, the commercial availability of the aryl boronic acids and their lower toxicity. Unfortunately, this method proved to be inconsistent, giving only some of the desired products, often in very low yields (<5%), along with homo-coupling of the iodovinyl estradiol as the major product. This observation led us to use our previously established Stille coupling strategy.

Coupling 17 α -E-tri-n-butylstannylvinyl estradiol **1** with the corresponding bromo/iodoheteroarenes gave the desired heteroarylvinyl estradiols **2b–g**, in acceptable overall yields (18–80%) with minimal by-products.²⁵ In some cases the product was accompanied by the homo-coupled vinyl estradiol dimer. ¹H-NMR spectrometry confirmed the anticipated E-stereochemistry (J= 16 Hz) while the chemical shifts of the C₂₀,C₂₁-protons reflected the electron donating/withdrawing character of the heteroarene. The observed chemical shifts ranged from 6.41–6.51 and 6.67–6.77 δ for the electron rich thienyl derivatives to 6.56–6.69 and 6.86–7.18 δ for the electron-deficient pyridyl and pyrimidinyl derivatives. Chromatography also demonstrated that the nitrogen-containing heterocyclic

arylvinyl estradiols were significantly more polar than the corresponding phenyl- and thienylvinyl estradiols.

Biological Evaluation

The new compounds **2b–g** and the parent 17 α -E-phenylvinyl estradiol **2a** were evaluated for estrogen receptor binding affinity using competitive binding of [³H]-estradiol for the ER α -LBD derived from *E. Coli*, and for biological potency using induction of alkaline phosphatase in Ishikawa cells.^{26–28} The results of these assays are shown in Table 1. As the data indicate, the parent compound, previously characterized, has ER α -LBD binding (RBA) and biological potency (RSA) that are approximately 10% that of estradiol. Introduction of one nitrogen (2-/3-/4-pyridyl), two nitrogens (5-pyrimidinyl) or a sulfur (2/3-thienyl) into the ring produced a significant (2.5–10-fold) reduction in the RBA values. The effect of heteroatom substitution on efficacy was different as only the thienyl derivatives exhibited significant stimulatory activity in this assay (RSA = 2.5%) and were full agonists, whereas the aza-heterocyclic derivatives were essentially inactive (RSA = 0.1–0.4%). None of the compounds inhibited the stimulatory activity of estradiol.

In previous studies we noted that the nature and position of substituents on the phenyl ring had a significant effect on the ability of the ligand to bind to the ER α -LBD. In this study we selected heterocyclic arenes that are essentially isosteric to benzene and differ primarily in their electronic character. The two thienyl derivatives are more electron rich than the phenyl analog whereas the pyridyl derivatives are electron deficient, and have a weakly basic nitrogen (pKa ~5.2–5.5) oriented toward the ortho (2-), meta- (3-) or para (4-) position.^{29,30} The pyrimidinyl derivative is even more electron deficient and has its weakly basic nitrogens (pKa ~ 1.3–2.0) symmetrically oriented toward the meta- (3-,5-) positions. One of the effects of substitution that was observed during the purification of the products was the increased polarity of the nitrogen-containing heterocyclic derivatives. The thienyl derivatives have calculated total polar surface area (tPSA) values very similar to the phenyl parent compound (~40.5), whereas the pyridine and pyrimidine derivatives have significantly higher values (tPSA ~52.8 and 65.2 respectively). This is also reflected in the lower cLogP values for the nitrogen heterocycles (4.65 and 3.69) compared to the thienyl and phenyl analogs (5.69 and 6.15). These properties should be reflected in the interactions between the ligand and the ER α -LBD. The induced binding pocket in which the arylvinyl group lies is bounded by three methionine residues (Met-342,-343,-421) and one phenylalanine residue (Phe-425).²⁴ Repulsive interactions between the electron pairs of the heteroarenes and those present in methionines may partially explain the reduced binding affinity observed for the new derivatives. In addition, interactions between the π -electrons of the heteroarenes and the adjacent phenylalanine are possible but not likely given the distance and orientation of the two aromatic rings. Although such interactions have only a modest effect on affinity (RBA), it appears that the increased polarity (elevated tPSA, decreased cLogP, and basicity) of the nitrogen heterocycles compared to the lipophilic thienyl and phenyl derivatives results in a significantly lower ability of the ligands to stimulate the receptor (decreased RSA compared to RBA). This may be due to weakening the stability of the complex with respect to generating downstream responses. In previous studies we observed that the introduction of an 11 β -methoxy group had little effect on overall binding affinity of steroidal estrogens, yet dramatically increased agonist activity, presumably by influencing the stability of receptor-coactivator complexes. In this case, we may be observing the opposite effect, where the aza-arenes may subtly weaken the internal forces that favor co-activator binding, without generating major changes in ligand binding affinity.

In summary, we demonstrated the preparation of a series of novel heterocyclic analogs of the parent 17 α -phenylvinyl estradiol using Stille coupling methods. Biological assays indicated that the introduction of the heteroatom had the general effect of reducing binding affinity, however, the ability of the aza-arene derivatives to activate the receptor was dramatically reduced compared to the thienyl and the parent phenyl derivatives. The results suggest that interactions between the electron pairs on the aryl group and the peptide residues in the ligand binding pocket are important factors in correlating chemical structures with biological responses within the estrogen receptor field.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and Notes

1. Tsai MJ, O'Malley BW. Annu. Rev. Biochem. 1994; 63:451. [PubMed: 7979245]
2. Nilsson S, Gustafsson J-A. Crit. Rev. Biochem. Mol. Biol. 2002; 37:1. [PubMed: 11905545]
3. Evans RM. Science. 1988; 240:889. [PubMed: 3283939]
4. Katzenellenbogen BS, Choi I, Delage-Mourroux R, Ediger TR, Martini PGV, Montano M, Sun J, Weis K, Katzenellenbogen JA. J. Steroid Biochem. Mol. Biol. 2000; 74:279. [PubMed: 11162936]
5. Duterte M, Smith CL. J. Pharmacol. Exp. Thera. 2000; 295:431.
6. Hart LL, Davie JR. Biochem. Cell Biol. 2002; 80:335. [PubMed: 12123286]
7. McKenna NJ, O'Malley BW. J. Steroid Biochem. Mol. Biol. 2000; 74:351. [PubMed: 11162944]
8. Krishnan V, Heath H, Bryant HU. Vit. Horm. 2001; 60:123.
9. Macgregor JJ, Jordan VC. Pharmacol Rev. 1998; 50:151. [PubMed: 9647865]
10. Jordan VC. J. Med. Chem. 2003; 46:883. [PubMed: 12620065]
11. Jordan VC. J. Med. Chem. 2003; 46:1081. [PubMed: 12646017]
12. Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson JA, Carlquist M. Nature. 1997; 389:753. [PubMed: 9338790]
13. Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL. Cell. 1998; 95:927. [PubMed: 9875847]
14. Tanenbaum DM, Wang Y, Williams SP, Sigler PB. Proc. Natl. Acad. Sci. U.S.A. 1998; 95:5998. [PubMed: 9600906]
15. Pike ACW, Brzozowski AM, Hubbard RE, Bonn T, Thorsell A-G, Engstrom O, Ljunggren J, Gustafsson J-A, Carlquist M. EMBO J. 1999; 18:4608. [PubMed: 10469641]
16. Napolitano E, Fiaschi R, Hanson RN. J. Med. Chem. 1991; 34:2754. [PubMed: 1895295]
17. Napolitano E, Fiaschi R, Herman LW, Hanson RN. Steroids. 1996; 61:384. [PubMed: 8776802]
18. Hanson RN, Herman LW, Fiaschi R, Napolitano E. Steroids. 1996; 61:718. [PubMed: 8987141]
19. Hanson RN, Lee CY, Friel CJ, Dilis R, Hughes A, DeSombre ER. J. Med. Chem. 2003; 46:2865. [PubMed: 12825929]
20. Hanson RN, Friel CJ, Dilis R, Hughes A, DeSombre ER. J. Med. Chem. 48. 2005:4300. [PubMed: 15974584]
21. Mobley JA, L'Esperance JO, Wu M, Friel CJ, Hanson RN, Ho S-M. Mol. Cancer. Ther. 2004; 3:587. [PubMed: 15141016]
22. Hanson RN, Lee CY, Friel C, Hughes A, DeSombre ER. Steroids. 2003; 68:143. [PubMed: 12606005]

23. Hanson RN, Dilis R, Tongcharoensirikul P, Hughes A, DeSombre ER. J. Med. Chem. 2007; 50:472. [PubMed: 17266199]
24. Nettles KW, Bruning JB, Gil G, O'Neill EE, Nowak J, Hughes A, Kim Y, DeSombre ER, Dilis R, Hanson RN. EMBO Reports. 2007; 8:563. [PubMed: 17468738]
25. General Synthetic Method. **17 α -E-(3-Pyridyl)-vinyl estradiol 2c**. 17 α -E-tri-n-butylstannylvinyl estradiol **1** (0.50 mmol, 0.293 g), 3-iodopyridine (1.50 mmol, 0.310 g), dried cesium fluoride (0.40 g), and 25 mg bis (tri-t-butylphosphine)palladium (0) were evacuated and purged with argon four times. Dry dioxane (3 mL) was added, the mixture was sealed under an argon atmosphere and heated at 80°C until the reaction was complete (monitored by TLC). The hot reaction mixture was filtered and the residue was washed with acetone. The filtrate was evaporated to dryness and the product was purified by automated flash chromatography on silica gel using hexane-ethyl acetate (step gradient) as the eluent. The fractions containing pure product were combined and evaporated to yield 34 mg (0.09mmol, 18% yield). The product was characterized by ¹H-, ¹³C-NMR, HRMS, and elemental analysis. Characterization of the less polar major component identified the material as the vinyl estradiol homodimer.
26. Labaree DC, Shang J, Harris HA, O'Connor C, Reynolds TY, Hochberg RB. J. Med. Chem. 2003; 46:1886. [PubMed: 12723952]
27. Green S, Walter P, Kumar V, Krust A, Bonert JM, Argos P, Chambon P. Nature. 1986; 320:134. [PubMed: 3754034]
28. Littlefield BA, Gursipide E, Markiewicz L, McKinley B, Hochberg RB. Endocrinology. 1990; 127:2757. [PubMed: 2249627]
29. Bird, CW.; Cheeseman, GWH. Comprehensive Heterocyclic Chemistry. Katritzky, AR.; Rees, CW., editors. Vol. Volume 4. Pergamon Press; New York, NY: 1984. p. 1-39.
30. Johnson, CD. Comprehensive Heterocyclic Chemistry. Katritzky, AR.; Rees, CW., editors. Vol. Volume 2. New York, NY: Pergamon Press; 1984. p. 99-164.

Reagents and Conditions

(i) Ar-X, [(t-Bu)₃P]₄Pd(0), CsF, dioxane, 65-100° C, 2-4 h

Scheme 1.

Synthesis of (hetero)aryl vinyl estradiols **2a-g**

Table 1

Relative Binding Affinity (RBA) and Relative Stimulatory Activity (RSA) for (Hetero)Arylvinyl Estradiols **2a–g**

2a–g

Compound	Ar=	RBA	RSA
2a	Phenyl	10.3±2.9	9.5±2.5
2b	2-Pyridyl	1.5±0.8	0.1±0.07
2c	3-Pyridyl	4.0±1.0	0.25±0.07
2d	4-Pyridyl	2.5±1.0	0.1±0.1
2e	5-Pyrimidinyl	0.8±0.3	0.4±0.2
2f	2-Thienyl	2.7±1.2	2.5±0.1
2g	3-Thienyl	2.9±0.4	2.5±0.8

RBA = $100 \times [E]/[C]$ where [E] is the concentration of unlabeled estradiol necessary to reduce the specific binding of tritiated estradiol to the ER α -HBD by 50% and [C] is the concentration of the competitive ligand necessary to reduce specific binding by 50%. The RBA of estradiol is 100% at 25 °C. Curves for ligand and estradiol had correlation coefficients >95%. RSA = Relative Stimulatory activity compared to E₂ = 100%, in stimulation of alkaline phosphatase (AlkP) in the Ishikawa cell line. EC₅₀ for E₂ = 0.9 ± 0.2 nM.